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HYPOPHYSIAL CONTROL OF CUTANEOUS PIGMENTATION IN AN ELASMOBRANCH FISH [1]

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In a study of the effects of ablation of various parts of the brain of the dogfish, *Mustelis canis*, it was noticed that certain operated individuals became, in the course of the first few post-operative hours, lighter in color than their fellows. The degree of paling was such as to render the affected animal distinctly lighter than any normal dogfish of this species that we have seen. Inspection of the cranial contents of the operated fish showed that whenever the albinous appearance was produced the pituitary body had been extirpated in the intracranial intervention. On the other hand, the hypophysis was invariably intact in those operated fish which retained their normal dark grayish color. Quite naturally we were led to suspect that the dogfish possesses an hypophysial control of its cutaneous pigmentation similar to that which has been so clearly established for the amphibia by P. E. Smith (1916), Allen (1917, 1920), Atwell (1919), Rehberg and Krogh (Krogh, 1922) and Hogben and his collaborators (Hogben, 1924). It is a curious fact that, so far as we have been able to ascertain, no experimental investigation of chromatic function in the elasmobranch group has ever been made, although these animals possess dermal chromatophores (melanophores and xanthophores) not unlike those seen in the frog (Daniel, 1922).

That pituitary removal alone is followed by the development of pallor was shown in a series of hypophysectomies performed on 40 dogfish. The operation is easily carried out through a buccal approach.

[1] A report of results obtained in an investigation carried out in the Course in Physiology at the Marine Biological Laboratory, Woods Hole, during the summer of 1931.

Method

The fish was tied down in the dorsal position on a small shark board and a stream of sea water introduced by means of a tube into the pharyngeal region to maintain respiration (under these circumstances normal breathing movements continue). After incision and retraction of the mucous membrane the base of the skull was lightly scraped along the midline at the level of the pupils of the eyes. This brings to view through the translucent cartilage the chiasma, hypothalami, and posterior hypophysis. A medial opening was then cut in the skull between the chiasma and the posterior lobe. This exposes the elongated anterior lobe of the pituitary which lies in the depression between the hypothalami and extends forward nearly to the chiasma. The width of the exposure was such as to lay bare the medial half of each ventral hypothalamic surface. So much may be done without the slightest hemorrhage. But it was found that extension of the skull defect backward so as to expose merely the cranial aspect of the large bifid neuro-intermediate or posterior lobe resulted in profuse bleeding. Therefore this structure was carefully scooped out by means of a small curette introduced through the limited opening. With a little care this can be done without provoking hemorrhage and with minimal injury to the base of the brain. The anterior lobe may be left intact or it may be picked up with forceps, separated from the overlying nervous tissue, and so extirpated with very little damage to the hypothalamus. No attempt was made to close the wound. Provided the base of the brain had not been severely traumatized, the operated fish swam around normally and showed a general behavior indistinguishable from that of unoperated specimens. They usually succumbed after three or four days with loss of righting reactions and dyspnea as premonitory symptoms. A few lived as long as a week, which is about the average length of life of unoperated specimens kept under laboratory conditions. It seems likely that death was often induced by cerebral injury, some degree of cerebral herniation being the usual finding at autopsy. We have not been disturbed by this rather crude postoperative situation. It is apparently without significance for the specific problem in hand, namely, the experimental analysis of pituitary control of cutaneous pigmentation, for injury or herniation of the hypothalamic region without removal of the hypophysis never gave rise to the slightest pallor. Several animals survived such a condition several days and retained the same shade as normal control animals.

Results

Complete removal of the pituitary body (30 experiments) always resulted in pallor. Slight paling was first noticeable about thirty minutes after pituitary ablation and after three hours the change was generally very marked, but maximal pallor was not attained until about the twelfth postoperative hour. It was our practice to select pairs of fish having the same degree of pigmentation, hypophysectomize one and retain the other for comparison. Plate 1 is a photograph of two such fish taken twenty-four hours after complete hypophysectomy in one. It shows clearly the extreme pallor characteristic of the animal without hypophysis. That the pallor is due to "contraction" of the dermal melanophores is shown in Plates 3 and 4, which are microphotographs of homologous pieces of skin taken from the same fish just before and nineteen hours after hypophysectomy. As shown in Plate 3 the melanophores of the normal fish are in a state of considerable expansion, while Plate 4 indicates clearly that the pallor developing after pituitary removal is the result of the assumption by the melanophores of a more or less rounded contour. Were it not for the outlines of several placoid scales one might easily mistake these plates for microphotographs of the skin of a normal and a hypophysectomized frog or axolotl.

Removal of the anterior lobe alone was done in five dogfish and these survived the operation for several days without showing the slightest change in cutaneous pigmentation. Nor did leaving the anterior lobe intact impede in any way the development of the pallor ensuing upon removal of the posterior lobe. As already mentioned, severe traumatization of the hypothalamus was found to be without influence on pigmentation provided the posterior lobe of the pituitary was not ablated. In four fish the anterior lobe and hypothalamic protuberances were exposed in the usual way, transverse incisions made in these structures to a depth of one or two millimeters and much of the substance of the hypothalamus pulled out with forceps. Two of these animals survived thirty hours without at any time presenting the slightest change in hue. The other two, whose color also remained unchanged, were subjected to posterior pituitary removal after twenty-four hours whereupon there ensued a pallor which developed at the usual rate and to the usual extent.

Further evidence for an hypophysial control of the cutaneous melanophores was sought in a study of the effects of pituitary preparations when injected into pale hypophysectomized dogfish. Each of five pale specimens received subcutaneously a crushed dogfish posterior lobe suspended in one or two cubic centimeters of sea water. A

distinct general darkening appeared within the first three minutes and after an hour these fish were indistinguishable from the darkest normal animals. Then in the course of the next five or six hours they gradually returned to the albinous condition. Injections of similar suspensions of cerebellum, skeletal muscle, and pancreas from normal dogfish were entirely without effect. In three experiments it was found that administration of crushed anterior lobe resulted in a moderate darkening which was slower to develop and which disappeared more rapidly than the more intense darkening caused by an equal quantity of crushed posterior lobe. In one pair of pale fish injection into one of a crushed posterior lobe fragment of about half the size of the anterior lobe produced an immediate darkening which reached its maximum in one hour, while injection of the crushed anterior lobes of two fish into the other produced a moderate darkening which was slow to develop and had a very short duration. These results together with those of the ablation experiments indicate that the posterior lobe is the chief source of the melanophore-expanding substance or secretion. The activity of the anterior lobe material may well have been due to diffusion into it of active substance of posterior lobe origin. Shortage of fish prevented our exploring this question further.

Extracts of several posterior lobes were made. Immediately after its removal from a living dogfish the lobe was crushed, ground up, stirred and shaken in sea water, and the resulting suspension filtered. After appropriate dilution with sea water the clear filtrate was tested by injection of varying amounts into thoroughly pale hypophysectomized fish of the same size as the donor of the lobe. It was found that an amount of filtrate corresponding to one twenty-fifth of one posterior lobe was necessary to produce a noticeable darkening while it required from one-tenth to one-seventh of a filtrate to make such a fish as dark as normal controls. It is unlikely that such simple extracts as these represent the full potency of the glands. Hogben (1924) states that the amount of melanophore stimulant in the pituitary of one frog is sufficient to darken the skin of fifty-six animals of the same species.

The posterior pituitary preparations "pituitrin" (Parke, Davis & Co.) and "infundin" (Burroughs, Wellcome & Co.) produced darkening in pale pituitaryless fish when given subcutaneously or intramuscularly. Plate 2 is a photograph of the pair of fish shown in Plate 1. It was taken one hour after the hypophysectomized individual had received a subcutaneous injection of 0.4 cc. of "obstetrical pituitrin," an amount equivalent to four international oxytocic units. It can be seen that this dose had darkened the animal almost to its preoperative

shade. In the case of several pale hypophysectomized fish, twenty to thirty inches in length, it was found that a subcutaneous dose of about 0.5 cc. (5 international units) of "obstetrical pituitrin" was required to produce in them a darkness equal to that of normal controls. It seemed interesting to test the action on elasmobranch melanophores of the two active principles separated from pituitary extracts by Kamm and his associates and now supplied by Parke, Davis and Co. under the names "pitressin" and "pitocin." It was found that 0.25 cc. (5 pressor units) of "pitressin" was capable of producing approximately the same effect as 0.5 cc. (5 international units) of "pituitrin." On the other hand 0.5 cc. (5 international units) of "pitocin" had no effect whatever on pale individuals of the same size. When twice this quantity of "pitocin" was injected there occurred a slight darkening which reached its maximum much more slowly and disappeared more rapidly than the reaction to "pitressin" or "pituitrin." Since "pitocin" contains one-half unit of pressor activity per cubic centimeter, it is probable that this mild positive result was due to the pressor substance present. These results are in general agreement with those of L. W. Rowe (1928), who reported that "vasopressin" ("pitressin") but not "oxytocin" ("pitocin") caused expansion of frog melanophores, a finding which is in contradiction to the inference of Hogben and Winton (1922*b*) that the uterine and melanophore stimulants of pituitary extracts are identical.

We had hoped to secure evidence of the presence of melanophore-expanding material in the circulating blood, but shortage of dogfish during the latter part of the summer made this impossible. Indirect evidence that pituitary substance acts directly on the dermal melanophores was, however, secured in our observation, often repeated, that the melanophores of small isolated pieces of skin from pale dogfish expanded markedly when such skin fragments were placed in dilute sea-water solutions of "pituitrin" or "pitressin" or in suspensions or extracts of dogfish posterior lobe. When such pieces were immersed in sea water alone the melanophores remained contracted. The expanded melanophores of bits of skin from normal fish gradually contract when placed in sea water. Addition of "pituitrin," etc., prevented this slow contraction. Equivalent solutions of "pitocin" had no action on the melanophores of skin fragments.

Discussion

The results presented in the foregoing section give abundant proof that the normal dark grayish color of the dogfish is maintained through the agency of the hypophysis. Apparently this organ continuously

delivers to the blood stream a quantity of melanophore stimulant capable of maintaining the rather widely expanded state of the cutaneous melanophores to which the normal color of the fish is due. The experimental findings strongly suggest that this humoral agent has its chief, if not its sole, origin in the posterior lobe. This portion of the gland is more correctly termed the neuro-intermediate lobe. Apart from a rather small extension of neuroglia fibers from the border of the infundibular cavity it is wholly composed of a highly vascular mass of basophil cells which, on the basis of embryological as well as histological observations, obviously constitutes the homologue of the pars intermedia of higher forms (de Beer, 1926). The ventral lobe of the selachian hypophysis which is attached dorsally to the neuro-intermediate lobe and ventrally to the skull and which, according to de Beer (1926), is composed largely but not entirely of basophil cells, has not been taken into separate account in our experiments. In all of our posterior lobe extirpations it was doubtless removed. It would seem highly probable that the melanophore-expanding substance originates in the pars intermedia. There is excellent evidence that such is the case in the amphibia. We know that there too anterior lobe removal is not followed by pallor (Hogben and Winton, 1923) and that while the melanophore stimulant is not lacking from extracts of pars anterior and pars nervosa of beef glands, there is more of it per gram of dried tissue in the pars intermedia than in the other two portions of the gland (Hogben and Winton, 1922*a*, 1922*b*). Some further confirmation of this point comes from Atwell's report (1919) that albinous tadpoles darken as a result of melanophore expansion when placed in dilute extracts of beef pars intermedia. But by far the most conclusive results have been those obtained by B. M. Allen (1920) in his study of the effects of transplantation of different parts of the hypophysis of the adult frog (*Rana pipiens*) into tadpoles from which the pars buccalis had been removed (anlage of pars nervosa left intact) and which, as a result, exhibited pallor as well as failure to metamorphose and retardation of growth. The operated larvae which received anterior lobe transplants showed "not the slightest tendency to return to the original black color except for a slight tendency at the beginning," although this treatment caused marked acceleration of growth. On the other hand, the albinous tadpoles regained their dark color when the intermediate lobe was engrafted into them. In a recent communication (1930) Allen reports experiments in confirmation of his earlier results and shows that transplants of pars nervosa do not cause pigmentary effects. Allen's work is a most significant contribution to the problem

of localization of pituitary function. Although it presents the only unequivocal evidence for the precise origin of the amphibian melanophore stimulant, it is not referred to in Hogben's monograph (1924). Shortly after the appearance of Allen's paper, Swingle (1921) reported that intraperitoneal transplantations of pars intermedia from various frogs into bullfrog larvæ caused darkening of the skin. It can be concluded that in the frog the pigmentary hormone is produced only by the pars intermedia and our own findings lend support to the view that such is the case in the elasmobranch.

It is well known that amphibia exhibit a rhythm of color response which depends on a balance of such environmental factors as humidity, temperature, oxygen supply, and illumination (Hogben, 1924). In the majority of amphibia light causes pallor while its absence promotes darkening. We have found that dogfish taken from a moderately illuminated tank paled markedly when placed in a brightly illuminated tank, while other fish originally of the same shade and from the same tank darkened perceptibly when placed in a tank from which nearly all light was excluded. These changes require several hours. They cannot, of course, be referred to changes in humidity and we were at some pains to be sure that during these tests the temperature and aëration of the tanks were equal. Segments of illuminated fish which were covered by bands of adhesive plaster did not remain dark. We conclude, therefore, that light causes pallor by a general rather than a local action, and since pale hypophysectomized fish remained pale when kept in darkened tanks we can assume that these pigmentary changes evoked by changes in illumination are the results of variations in pituitary activity.

Hogben (1924) is strongly of the opinion that regulation of color response in the amphibia by melanophore contraction and expansion is correlated with a fluctuating pituitary secretion. He believes this to be an adequate explanation of all the pigmentary phenomena seen in these animals and he is inclined completely to discount all assumptions of a direct nervous control of amphibian melanophores. He suggests that such chromatic effects as have been observed following nerve section or stimulation or drug administration have been due to vasomotor changes. Since it is clearly established that adrenalin injected intravenously causes pallor in the frog we made some effort to determine whether injection of this substance has any influence on the pigmentation of the dogfish. In a few preliminary experiments we gained the impression that large subcutaneous injections of adrenalin caused slight paling. Subsequently, at a time when the supply of fish had greatly diminished, we tested the effect of injections made

directly into the heart chambers. In collaboration with Dr. René Gayet, who kindly offered to share a number of fish with us, it was found that 1 cc. of a 1 : 1000 solution of adrenalin chloride evoked a paling which usually became noticeable after from five to ten minutes, sometimes not until after fifteen minutes, progressed to a maximum in about an hour, and persisted for approximately two hours. In one case the pallor was maximal; in the others it was not. Direct microscopical examination showed that in these cases the melanophores had actually undergone contraction. But the inadequate number of experiments performed makes it hazardous to venture an inference. Yet the comparative sluggishness of the responses provoked by these enormous doses which obviously produced a state of shock does not strongly suggest that the dogfish possesses an adrenal or sympathetic control. It may well have been that vasoconstriction of cutaneous vessels led to an asphyxiation and consequent contraction of the melanophores. Because of scarcity of material we were obliged to postpone to a later occasion an adequate investigation of the interesting question of a possible extra-pituitary control of elasmobranch chromatophores.

Summary

1. Complete hypophysectomy in the dogfish, *Mustelis canis*, results in pallor of the skin. This same result follows removal of the posterior (neuro-intermediate) lobe alone. Extirpation of the anterior lobe does not result in paling. Severe traumatization of the hypothalamus without destruction of the posterior lobe of the hypophysis has no influence on cutaneous pigmentation.

2. The pallor following hypophysectomy is due to "contraction" of the cutaneous melanophores which are normally in a state of considerable "expansion."

3. Suspensions or extracts of dogfish posterior lobe and the commercial posterior pituitary preparations, "pituitrin," "infundin," and "pitressin" produce melanophore expansion and consequent darkening when injected subcutaneously or intramuscularly into pale hypophysectomized dogfish. Suspensions of dogfish cerebellum, skeletal muscle, and pancreas have no effect upon pigmentation. The oxytocic principle of the preparation, "pitocin," does not produce melanophore expansion in hypophysectomized fish. Administration of suspensions of dogfish anterior lobe cause a relatively weak darkening of pale fish. The melanophores of isolated pieces of dogfish skin react to these various substances in the same way as do the melanophores of fish receiving them by injection.

4. Bright illumination of normal dogfish for several hours causes a

PLATE 1. Photograph of a pair of dogfish, originally of the same shade, taken twenty-four hours after removal of the hypophysis in the animal on the right. Both animals had been kept in the same tank and the operated individual showed no abnormalities of behavior.